REMARKS

Claims 105 and 139 have been amended. Support for the amended claims can be found throughout the application as filed, e.g., at page 2, lines 26-27, page 27, lines 18-26, and page 39, line 27 to page 40, line 11. Claims 107 and 108 have been canceled. No new matter has been added. Claims 105, 106, and 109-171 are currently under examination.

Sequence Listing

Applicants hereby submit that the enclosures fulfill the requirements under 37 C.F.R. §1.821-1.825.

The specification has been amended to insert a SEQ ID NO reference to the human GCB protein and gene sequences which were specifically incorporated by reference on page 27, lines 15-17, and page 56, line 6, of the original specification and to insert a paper copy of the Sequence Listing. The relevant material is inserted by the above amendment to the specification and by the corresponding sequence listing. An In re Hawkins declaration executed by Applicant's attorney is submitted herewith which verifies that the amendatory material consists of the material incorporated by reference in the application. No new matter has been added.

The Invention

The present claims under examination are directed to a method of producing a high mannose glucocerebrosidase (hmGCB). The method includes: providing a cell capable of expressing a human GCB; contacting the cell with a substance (e.g., kifunensine) such that the removal of at least one mannose residue distal to the pentasaccharide core of the precursor oligosaccharide of GCB is prevented; allowing the cell to produce hmGCB, and harvesting the hmGCB.

N-linked oligosaccharide processing occurs, in general, as disclosed in the specification at page 31, line 23, to page 32, line 25:

> N-linked oligosaccharide processing can be divided into three stages: [1] removal [from the precursor oligosaccharide] of the three glucose residues, [2] removal of a variable number of mannose residues, and [3] addition of various sugar residues to the resulting trimmed core.

> The removal of the glucose residues in the first stage of processing involves removal of all three glucose residues to generate N-linked **$Man_9GlcNAc_2$**. . . . Processing normally continues to the second stage with removal of mannose residues.
>
> Four of the mannose residues of the $Man_9GlcNAc_2$ moiety are bound by α 1,2 linkages. Up to four of these α 1,2-linked mannose residues can be removed by mannosidase IA, IB and IC to generate N-linked $Man_{5-8}GlcNAc_2$[. . .]
>
> Mannosidase II can then complete the trimming phase of the processing pathway by removing two mannose residues to generate a protein-linked oligosaccharide which contains within it a $Man_3GlcNAc_2$, the "pentasaccharide core"[. . . .]After the trimming phase, monosaccharides are sequentially added to the growing oligosaccharide chain by a series of membrane-bound Golgi glycosyltransferases [. . .] The cooperative action of these glycosyltransferases produces a diverse family of structures collectively referred to as "complex" oligosaccharides.

In human cells, oligosaccharide processing of GCB results in a GCB glycoprotein whose glycan chains are predominantly of the complex type (see, e.g., page 27, lines 18-26, of the specification). That is, 3 mannoses are present in the pentasaccharide core, but are internal to molecules of N-acetylneuraminic acid, galactose, and N-acetylglucosamine in the complex glycan chains that are added to the molecule during the third stage of processing, after the removal of the mannose residues that occur during the second stage, discussed above. The inventors have developed the claimed methods to prevent "removal of at least one mannose residue distal to the pentasaccharide core of the precursor oligosaccharide of GCB," i.e., to prevent the removal of one or more of the distal mannose residues of **$Man_9GlcNAc_2$** that occurs during the second stage of normal N-linked oligosaccharide processing described above, thereby providing GCB with high mannose chains (hmGCB). As quoted above from the specification, "up to four of these α 1,2-linked mannose residues can be removed by mannosidase IA, IB and IC to generate N-linked $Man_{5-8}GlcNAc_2$." Accordingly, with respect to claim 105 and its dependencies, if removal of all four of these outer mannose residues is prevented by kifunensine (a mannosidase I inhibitor), the resulting GCB will retain all 9 mannose residues (Man_9); if removal of three of the four outer mannose residues is prevented by kifunensine, the resulting

GCB will retain 8 mannose residues (Man_8), etc. With regard to claim 139 (which is not limited to kifunensine) contacting the cell with a substance which prevents the removal of at least one mannose residue distal to the $Man_3GlcNAc_2$ pentasaccharide core of a precursor oligosaccharide of GCB will result in GCB having 4 or more mannose residues. Such high mannose GCB's can effectively target the mannose receptors on reticuloendothelial cells and be efficiently delivered to the lysosomes, e.g., for the treatment of Gaucher's disease.

Rejections Under 35 U.S.C. § 112, First Paragraph

I. Written Description

Claims 105-171 are rejected under 35 U.S.C. § 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention." This rejection is respectfully traversed.

The Examiner summarizes the claims as follows:

> These claims are directed to a method of use of a cell comprising a genus of a glucocerebrosidase (GCB), with claims 127 and 128 reciting an exogenous nucleic acid sequences comprising a GCB coding region, with claim 128 further limiting to an exogenous regulatory sequence. Claims 139-171 recite a cell comprising an exogenous regulatory sequence without specifying whether a GCB coding sequence is exogenous or endogenous. Therefore, the claims comprise the genus of cells both naturally occurring and recombinant comprising either endogenous or exogenous GCB from any source.

At the outset, Applicants point out that claim 139 explicitly recites that "an exogenous regulatory sequence has been introduced such that the regulatory sequence regulates the expression of an endogenous GCB coding region." Thus, contrary to the Examiner's statement, claim 139 and dependent claims 140-171 do specify that the GCB coding region is endogenous. The Examiner provides the following basis for the rejection.

> The specification teaches only a single representative species of such cells, human HT-1080 cells that produce Gene-Activated GCB (GA-GCB). Assuming that said cells express a human GCB, the specification does not teach an amino acid structure of said gene whereas it is known that humans have various allelic variants of GCB genes. . . .Furthermore, there is no description of how many

> carbohydrate chains the human unmodified GCB has and what is their carbohydrate composition. The art teaches that the oligosaccharide chains of animal glycoproteins are attached via N-glycosidic linkage to an asparagine (Asn) residue. . . Two, three or four oligosaccharide chains are attached to the invariant core pentasaccharide $Man_3GlcNAc_2$. . . .Therefore, the genus of GCBs expressed by cells used in the instant methods, encompasses GCBs from various sources, i.e., having different amino acid structures and different carbohydrate compositions. Moreover, the specification fails to describe any other representative species by any identifying characteristics or properties other than the functionality of expressing a GCB. With regard to claims 127, 128 and 139-171 reciting an exogenous coding and/or regulatory sequence, there is no description of any GCB gene. Specifically, there is no description of any GCB gene into which said regulatory sequence can be integrated. Given this lack of description of representative species encompassed by the genus of the claims, the specification fails to sufficiently describe the claimed invention in such full, clear, concise and exact terms that skilled artisan would recognize that applicants were in possession of the claimed invention.

This basis for rejection has been met in part and is respectfully traversed in part. The independent claims (claims 105 and 139) have been amended to recite cells expressing a human GCB. Claim 105 recites a method of producing a high mannose glucocerebrosidase (hmGCB). The method includes: providing a cell capable of expressing a human GCB; contacting the cell with kifunensine such that the removal of at least one mannose residue distal to the $Man_3GlcNAc_2$ pentasaccharide core of the precursor oligosaccharide of GCB is prevented; allowing the cell to produce hmGCB, and harvesting the GCB. Claim 139 is directed to a method of producing hmGCB. The method includes providing a human cell into which a nucleic acid sequence comprising an exogenous regulatory sequence has been introduced such that the regulatory sequence regulates the expression of an endogenous GCB coding region; contacting the cell with a substance which prevents the removal of at least one mannose residue distal to the $Man_3GlcNAc_2$ pentasaccharide core of a precursor oligosaccharide of GCB; and allowing the cell to produce hmGCB. For at least the reasons discussed below, the application provides sufficient written description of the present claims.

Contrary to the Examiner's statement in the above-quoted passage of the office action, the application provides a detailed description of the identifying characteristics and properties of human GCB, including how many carbohydrate chains the human unmodified GCB has and

what is their carbohydrate composition. In particular, the specification at page 27, lines 18-26, provides as follows.

> Mature human GCB has five potential N-linked glycosylation sites at Asn-19, Asn-59, Asn-146, Asn-270, and Asn-462. Glycosylation occurs at four of the five sites in human tissue derived GCB (Erickson et al. (1985) *J. Biol. Chem.* 260:14319-14324). Studies employing site-directed mutagenesis have demonstrated that the site at Asn-462 is never occupied (Berg-Fussman et al. (1993) J. Biol. Chem. 268:14861-14866). Approximately 20% of the released glycan chains from human placental GCB were shown to be of the high mannose type containing up to seven mannose residues, whereas the majority of the glycan chains were of the complex type with sialylated biantennary and triantennary structures. (Takasaki et al. (1984) *J. Biol. Chem.* 259:10112-10117). (Emphasis added.)

Although the above paragraph refers specifically to human placental GCB, other human GCB's, such as GCB from human fibroblasts were known in the art at the time of filing to have a majority of complex type glycans. Indeed, Erickson et al., cited in the passage quoted above, indicate that all the glycans of human fibroblast GCB are complex type glycans. Therefore, an ordinary artisan would understand the term "human GCB" to describe a GCB with the particular structure and carbohydrate composition provided above, i.e., GCB having predominantly (or exclusively) complex glycan structures. Furthermore, the specification provides a fully representative example of the claimed methods using human HT-1080 cells genetically engineered to express the endogenous human GCB gene by gene activation (GA-GCB).[1] The Examiner is directed to page 53, lines 1-26 of the specification, which describes the experiment, and page 55, Table 3, which shows the results of the experiment. As can be seen in Table 3, 99% of the total GCB glycans from kifunensine-treated human GA-GCB cells were high mannose (hmGCB having 5 to 9 mannose residues per glycan chain on average), i.e., the removal of 1 to 4 mannose residues distal to the pentasaccharide core of the precursor oligosaccharide of GCB having been prevented, as recited in the claims. In contrast, as discussed above, glycans from unmodified GCB from untreated human GA-GCB cells are predominantly complex glycans (having only 3 mannose residues per glycan chain, internal to

[1] HT-1080 cells expressing GA-GCB are described in more detail below, in response to the Examiner's request for information.

molecules of N-acetylneuraminic acid, galactose, and N-acetylglucosamine). See, e.g., page 32, lines 19-25, describing the invariant core pentasaccharide $Man_3GlcNAc_2$.

In part, the Examiner appears to base the rejection on the alleged lack of description of the structure of the human GCB protein and, with regard to claims 127, 128 and 139-171, the human GCB gene. However, the protein and gene sequence of human GCB were known at the time of filing and are incorporated by reference in the application. See, e.g., Horowitz et al. (1989) *Genomics* 4:87-96 and Beutler et al. (1992) *Genomics* 12:795-800, cited at page 27, lines 15-17 of the specification; see also page 56, line 6, incorporating these references in their entirety. It would be a routine matter for one of skill in the art to express human GCB in a cell once the gene has been cloned.

Furthermore, the Examiner's concern that humans have allelic variants of the GCB gene is not relevant to the description of the claimed methods. The claimed methods work with any cell expressing a human GCB that has complex oligosaccharide chains. The known allelic variants of human GCB (as described by Beutler 1992, cited by the Examiner for the proposition that humans have allelic variants of GCB) are not known to affect the glycosylation sites or activity of GCB. Beutler 1992 states the following with regard to GCB alleles.

> Examination of 35 normal subjects and 51 Gaucher disease patients was consistent with the existence of only two major haplotypes. Two additional minor haplotypes were found, one in Africans and one in the white population. These represented additional mutations superimposed on the basic two haplotypes. (Beutler 1992 abstract)

Beutler does not disclose or suggest that the 2 major and 2 minor allelic variants of human GCB would have a different carbohydrate structure than that described in the specification, i.e., there is no reason to believe that different allelic variants of human GCB would not have a predominantly complex glycan structure, as described in the specification. As discussed above, the oligosaccharide structure of human GCB is sufficiently described in the present application. Thus, the application as filed is sufficient to reasonably convey to one of ordinary skill in the art that Applicants were in possession of the claimed methods. Accordingly, the instant application meets the requirements for written description

II. Enablement

Claims 107, 108, 119, 121-126, 141, 142, 151, 152 and 158-162 are rejected under 35U.S.C. §112, first paragraph, because "the specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention commensurate in scope with these claims. The Examiner provides the following reasoning:

> the specification, while being enabling for a cell comprising a human GCB remodeled to contain terminal mannose residues using mannosidase inhibitors, does not reasonably provide enablement for a GCB from any source and any structure and carbohydrate composition remodeled by preventing the removal of the specific number of mannose residues at specific locations.
>
> Without knowing the structure of GCB and mannosidase or a type of cell comprising them, it is unpredictable under which conditions the resulting "hmGCB" can be obtained. Without the knowledge of the kinetics of the specific reaction, it is impossible to set up the conditions under which removal of only one residue at the specific position will be prevented and the preparation comprising the requisite percent of the specific number of mannose residues will be obtained.
>
> ***
>
> The specification teaches the preparation of GCB comprising high mannose glycans such as $Man_9GlcNAc_2$ (31.2%), $Man_8GlcNAc_2$ (32%) and $Man_7GlcNAc_2$ (23.3%) using HT-1080 cells treated with kifunensine (page 55, Table 3)
>
> This result is specific for the specific reaction occurring in these cells under the specific experimental conditions. It is unpredictable as to how many mannose residues will retain on GCB present in a different type of cell under the same conditions. Thus, searching for conditions and substances that would lead to obtaining a hmGCB specifically quantitatively remodeled using any cell comprising any GCB is well outside the realm of routine experimentation and predictability in the art of success is extremely low.

This rejection has been met, in part, and is respectfully traversed, in part. The inventors have provided sufficient guidance for a skilled artisan to practice the full scope of the presently claimed methods.

This rejection has been met in part by amending the independent claims (105 and 139) to read "providing a cell which is capable of expressing a human glucocerebrosidase." The Examiner acknowledges that the specification "is enabling for a cell comprising a human GCB

remodeled to contain terminal mannose residues using mannosidase inhibitors." Claims 107 and 108 have been canceled.

The rejection is otherwise respectfully traversed. Claims 119, 121-126, 151, 152 and 158-162 recite specific numbers or percentages of mannose residues removed or retained. The specification provides detailed working examples of the claimed methods using human cells (HT-1080 cells) genetically engineered to express the endogenous human GCB gene by gene activation (GA-GCB). Unmodified GCB has 3 mannose residues (Man_3). Thus, when the removal of at least one mannose residue is prevented by the claimed methods, hmGCB having at least 4 mannose residues (Man_4 or greater) is produced (see, e.g., page 5, lines 4-11, of the specification). The Examiner is further directed to pages 51-56 of the specification, where the production, purification and characterization of hmGCB produced from cells expressing human GCB, using the claimed methods, is described in detail. Table 3 (page 55) shows the percentages of high mannose glycans obtained in an hmGCB preparation made by the claimed methods. The Examiner has provided absolutely no evidence that such results would not be predictably reproducible given the detailed guidance in the specification and the high level of skill in the art. This example is fully representative of results obtained using the claimed methods.

The results summarized in Table 3 show that the specific percentages of modified mannose residues recited in claims 119, 121-124 and 158-162 are enabled. With regard to claim 119 and 158, the results of Table 3 show that at least 99% of the hmGCB of the preparation has one or more carbohydrate chains in which the removal of one or more mannose residues distal to the pentasaccharide core has been prevented, i.e., at least 99% of the preparation has at least 4 mannose residues (Man_5 or higher). With regard to claims 121-123 and 159-161, the results show that 63.2% of the hmGCB of the preparation has at least 8 mannose residues (32% Man_8 plus 31.2% Man_9). With regard to claims 124 and 162, the results show that 97.7% of the hmGCB of the preparation has at least 6 mannose residues (adding the percentages of Man_6,

Man_7, Man_8 and Man_9). The specification provides detailed guidance on how these results were obtained. Therefore, claims 119, 121-124 and 158-162 are sufficiently enabled.

The Examiner also provides as follows:

> [C]laims 107 and 108 require the preventions of removal of α 1,3 mannose or α 1,6 mannose using kifunensine wherein the prior art teaches that kifunensine might prevent the removal of α 1,2 mannose. With regard to claims 141-142, the specification does not teach a compound that would specifically prevent the removal of α 1,3 mannose or α 1,6 mannose.

Claims 107 and 108 have been canceled. This aspect of the rejection is respectfully traversed with regard to claims 141 and 142. Class 2 mannosidases, described at page 34, lines 5-20, remove α1,3- and α1,6-linked mannoses. Contrary to the Examiner's statement, the specification teaches a number of substances that inhibit mannosidase 2, e.g., swainsonine, 6-deoxy-DIM, 6-deoxy-6-fluoro-DIM, mannostatin A, or combinations thereof (see, e.g., page 34, lines 17-20). Therefore, claims 141 and 142 are enabled.

With regard to claims 125-126 and 151-152, the Examiner states:

> the specification is enabled only for cells which comprise a class 2 processing enzyme of a known structure. Without the structure of a mannosidase, it is impossible to make a knockout gene or an antisense molecule.

This rejection is respectfully traversed. The specification provides detailed guidance for a skilled artisan to make a knockout or antisense cell for a mannosidase, see, e.g., pages 36-39 of the specification. Further, the specification provides as follows.

> There are at least three different human genes encoding related Golgi mannosidase I isoforms (IA, IB, and IC) with slightly different substrate specificities and tissue expression but all are capable of trimming four mannose residues from $Man_9GlcNAc_2$ glycans to form $Man_5GlcNAc_2$ (Tremblay et al. (July 27, 2000) *J. Biol. Chem.* [epub ahead of print]). They are located on chromosomes 6q22, 1p13, and 1p35-36 and their cDNA sequences are obtainable from GenBank as X74837, AF027156, and AF261655, respectively. (Specification, page 28, lines 6-12)
>
> As the coding strand sequences encoding various mannosidases are disclosed in, for example, Bause (1993) *Eur. J.* Biochem. 217(2):535-540; Gonzalez et al. (1999) *J. Biol. Chem.* 274(30):21375-21386; Misago et al. (1995) *Proc. Natl Acad. Sci. USA* 92(25):11766-11770; Tremblay et al. (1998) *Glycobiology*

8(6):585-595, Tremblay et al. (2000) *J. Biol. Chem.* Jul 27:[epub ahead of print], antisense nucleic acids can be designed according to the rules of Watson and Crick base pairing. (Specification at page 38, lines 11-16)

The references and Genbank Accession numbers cited above provide the coding sequence of, e.g., Man9-mannosidase from human kidney (Bause), human α1,2-mannosidase (Gonzalez), human α-mannosidase II and α-mannosidase IIx (Misago), human α1,2-mannosidase IA and IB (Tremblay 1998) and human Golgi α1, 2-mannosidase (IC) (Tremblay 2000). Making a knockout or antisense cell for a particular mannosidase is routine in the art once the coding or gene structure or sequence is known. Thus, by reference to the cited articles and sequences, applicants have provided the structure necessary for a skilled artisan to make antisense or knockout cells for a number of different human mannosidases. In addition, one of ordinary skill in the art would have available to her numerous other mannosidase sequences through routine searching of public sequence databases, e.g., Genbank. Thus, combined with the high level of skill in the art, applicants disclosure is sufficient to enable the use of mannosidase antisense or knockout cells as claimed.

Rejections Under 35 U.S.C. § 112, Second Paragraph

Claims 105-181 are rejected "as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention."

The Examiner asserts the following:

> The claims recite 'GCB'. Applicants refer to published structures for references. As an essential material those structures cannot be incorporated by reference (page 27). The specification does not define the number and composition of carbohydrate chains of an unmodified GCB. Further, the specification defines the term 'hmGCB' by non-limiting examples (page 15, line 25 through page 16, line 22) rendering the metes and bounds of the term unascertainable."

This grounds for the rejection is respectfully traversed. The present claims recite "a human GCB." As discussed above, the protein, coding and gene sequence of human GCB were known at the time of filing and are incorporated by reference in the application. See, e.g., Horowitz et al. (1989) Genomics 4:87-96 and Beutler et al. (1992) Genomics 12:795-800, cited at page 27, lines 15-17 of the specification. Accordingly, the scope of the term would be clear to

an ordinary artisan guided by the specification. However, to expedite prosecution, the specification has been amended to include the human GCB protein and gene sequences which were specifically incorporated by reference on page 27, lines 15-17, and page 56, line 6, of the specification. An In re Hawkins declaration executed by Applicant's attorney is submitted herewith which verifies that the amendatory material consists of the material incorporated by reference in the application. No new matter has been added.

In another aspect, the Examiner rejects claims 119 and 158 as being confusing in the recitation of the phrase "at least about 60% of the hmGCB of the preparation have one or more carbohydrate chains in which the removal of one or more mannose residues distal to the pentasaccharide core has been prevented." This grounds for rejection is traversed. As discussed above, the specification teaches that unmodified GCB has 3 mannose residues (Man_3). Thus, if "the removal of one or more mannose residues distal to the pentasaccharide core has been prevented," hmGCB having at least 4 mannose residues (Man_4 or greater) is produced. Therefore, it would be clear to one of ordinary skill in the art, guided by the specification, that claims 119 and 158 cover a preparation where at least 60% of the glycans in a hmGCB preparation have at least 4 mannose residues (Man_4 or greater) or greater. Such a preparation is exemplified by the hmGCB preparation characterized in Table 3, showing at least 97% of the hmGCB glycans being Man_5 or greater.

In another aspect, claims 121-123 and 159-161 are rejected on the grounds that "depending on the number of carbohydrate chains, the percent will vary rendering the metes and bounds of the claim unascertainable. It further renders unclear the difference in scope among these claims." This rejection is respectfully traversed. The claims are quite clear on their face, reciting specific percentages of hmGCB having at least 8 mannose residues. The Examiner's arguments for rejection seem to be based on a belief that human GCB structure is highly variable. To the contrary, as discussed above, human GCB has a specific, defined carbohydrate structure. Mature placental human GCB has four carbohydrate chains, 20% of which were shown to be of the high mannose type containing up to seven mannose residues, whereas the majority of the carbohydrate chains were of the complex type (having only 3 internal mannoses)

(see page 27, lines 18-26). The four carbohydrate chains of human fibroblast GCB are all complex type. The claimed methods can produce a hmGCB preparation having a certain percentage of glycans having at least 8 mannose residues (see, e.g., table 3, exemplifying a preparation having 63.2% glycans having at least 8 mannose residues). Thus, the metes and bounds of the claims are readily ascertainable by their plain meaning and when read in view of the specification.

In another aspect of the rejection, the metes and bounds of claims 124 and 162 are said to be unascertainable in the recitation of "at least about 80% or more of the carbohydrate chains of the hmGCB of the preparation have six or more mannose residues." This grounds for rejection is traversed. It would be clear to one of ordinary skill in the art reading the specification that hmGCB carbohydrates having "six or more mannose residues" are defined by Man_6, Man_7, Man_8 and Man_9 glycans. Thus, the preparation of claims 124 and 162 has 80% or more Man_6, Man_7, Man_8 and Man_9 glycans combined. Such a preparation is exemplified by the hmGCB preparation characterized in Table 3 (which has over 97% glycans having six or more mannose residues).

In light of the foregoing, applicants submit that the claims are clear when read in light of the specification.

Rejections Under 35 U.S.C. §103

In a first aspect of the rejection, claims 105, 106, 109-124, 132-135, 137 and 138 are rejected as unpatentable over Aerts et al. (Aerts) in view of Smith et al. (Smith). The present claims recite a method of producing a high mannose glucocerebrosidase (hmGCB). The method includes: providing a cell capable of expressing a human GCB; contacting the cell with kifunensine such that the removal of at least one mannose residue distal to the $Man_3GlcNAc_2$ pentasaccharide core of the precursor oligosaccharide of GCB is prevented; allowing the cell to produce hmGCB, and harvesting the GCB.

The Examiner characterizes the cited references as follows:

> [Aerts teaches] the production of high mannose GCB in the human monoblast cell line U937 using trimming inhibitors swainsonine or deoxymannojirimycin. They

> teach that swainsonine in an inhibitor of mannosidase II. . . .They teach the importance of hmGCB preparation for efficient routing of the GCB to the lysosome. . . .[Smith teaches] the method of preparing high mannose Man9(GlcNAc)2 glycopeptides by treating a cell with mannosidase inhibitors, deoxymannojirimycin or kifunensine.
>
> It would have been obvious to one of ordinary skill in the art at the time the invention was made to use either deoxymannojirimycin or kifunensine alone or in combination with swainsonine in a method taught by Aerts et al.

This rejection is respectfully traversed. To establish prima facie obviousness of a claimed invention, the prior art must teach or suggest all the limitations of the claims, and the motivation to arrive at the present invention and a reasonable expectation of success must be found in the prior art. In re Vaeck, 947 F.2d 488 (Fed. Cir. 1991). In this instance, a prima facie case of obviousness has not been made because the cited references, alone or in combination, fail to disclose or suggest all the limitations of the present claims. Nor does the art provide a motivation for a skilled artisan to arrive at the presently claimed methods. Indeed, the art in fact teaches away from the present claims, as discussed below.

Aerts uses cells treated with swainsonine or deoxymannojirimycin to study the role of carbohydrates on the biosynthesis and intracellular transport of glucocerebrosidase within the cell. Aerts does not disclose or suggest producing or harvesting hmGCB from such treated cells. If anything, Aerts suggests that making high mannose GCB is undesirable. To wit:

> Our findings suggest that in U937 cells the formation of complex type oligosaccharides in glucocerebrosidase is essential for efficient routing of the enzyme to the lysosome. . . .[The] presence of high mannose type and/or hybrid type oligosaccharide chains on glucocerebrosidase may prevent routing of the enzyme to the lysosomes.

Clearly, rather than "teach the importance of hmGCB preparation for efficient routing of the GCB to the lysosome" as the Examiner asserts, Aerts teaches that high mannose GCB would prevent proper routing to the lysosome, thereby teaching away from the claimed methods of making high mannose GCB.

Smith does not cure the deficiencies of Aerts. Smith describes treating a host cell with kifunensine to prevent binding of bacterial pili to the host cell. Smith does not mention

harvesting any high mannose containing polypeptides from a kifunensine treated host cell for any reason. Nor does Smith mention glucocerebrosidase at all. Therefore (and particularly in light of the teaching away of Aerts), neither Smith nor Aerts, either alone or in combination, teach or suggest the presently claimed methods.

In another aspect of the rejection, claims 105, 106, 109-118, 125, 126 and 129-171 are rejected as unpatentable over Aerts et al. in view of Smith et al. and further in view of Bergh et al. Aerts and Smith are discussed above. Bergh describes treating cells with inhibitors of mannosidase I or II. Bergh does not mention GCB at all. As such, Bergh does not add anything to the disclosures of the other cited references. Therefore, none of Smith, Aerts and/or Bergh, alone or as the examiner has combined the references, teach or suggest the presently claimed methods.

Claims 127 and 128 are rejected over Aerts et al. in view of Smith et al. and further in view of Friedman et al. Friedman discloses the expression of recombinant GCB. Friedman does not disclose or suggest using kifunensine to prevent the removal of at least one mannose distal to the pentasaccharide core of GCB. As such, Friedman does not cure the deficiencies of Aerts and Smith, discussed above.

In light of the foregoing, Applicants respectfully request that the rejection be withdrawn.

Request for Information

The Examiner has asked for information relating to "the embodiment of 'HT-1080 cells expressing Gene-Activated™ GCB (page 51, line 16)."

HT-1080 cells expressing Gene-Activated™ GCB are cells that have been genetically engineered to "turn on" the endogenous GCB gene. In the gene activation method, cells are transfected with a construct that, by targeted homologous recombination, replaces the regulatory region normally associated with the endogenous GCB gene in the cells with a regulatory sequence that causes the gene to be expressed at levels higher than evident in the corresponding nontransfected cells. This gene activation method is known in the art. For example, U.S. Patents 5,733,761 and 5,968,502, both of which issued before the filing date of the present application, teach methods of expressing a protein, including GCB, by gene activation. This is the method

used to make the HT-1080 cells expressing Gene-Activated™ GCB as disclosed in the present application.

Attached is a marked-up version of the changes being made by the current amendment.

Applicant asks that all claims be allowed. Please apply any charges or credits to Deposit Account No. 06-1050.

Respectfully submitted,

Date: October 18, 2002 Leda Tsirinas, Reg. No. 50,635

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Version with markings to show changes made

In the specification:

The paragraph starting at page 27, line 15 has been amended as follows.

Nucleotide sequence information is available for genes encoding glucocerebrosidase from various species. (See Horowitz et al. (1989) *Genomics* 4(1):87-96, disclosing the gene sequence (SEQ ID NO:1) and amino acid sequence (SEQ ID NO:2) of human glucocerebrosidase; Beutler et al. (1992) *Genomics* 12(4):795-800).

In the claims:

Claims 107 and 108 have been cancelled.

Claims 105 and 139 have been amended as follows:

105. (Amended) A method of producing a high mannose glucocerebrosidase (hmGCB) preparation, comprising:

providing a cell which is capable of expressing a human glucocerebrosidase (GCB);

contacting the cell with kifunensine such that the removal of at least one mannose residue distal to the pentasaccharide core of the precursor oligosaccharide of GCB is prevented; [and]

allowing the cell to produce hmGCB; and

harvesting the hmGCB from the cell or its culture media, to thereby produce an hmGCB preparation.

139. (Amended) A method of producing high mannose glucocerebrosidase (hmGCB), comprising:

providing a human cell into which a nucleic acid sequence comprising an exogenous regulatory sequence has been introduced such that the regulatory sequence regulates the expression of an endogenous GCB coding region;

contacting the cell with a substance which prevents the removal of at least one mannose residue distal to the pentasaccharide core of a precursor oligosaccharide of GCB; and

allowing the cell to produce hmGCB, to thereby produce an hmGCB preparation.